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STUDIES IN SWINE OF ASIAN INFLUENZA VIRUS

GORDON D. WALLACE, D.V.M. *
R. E. KISSLING, D.V.M., M.S.

Communicable Disease Center, Public Health Service,
US Department of Health, Education, and Welfare, Montgomery, Ala., USA

SYNOPSIS

This article reports on experiments with 6- to 8-week-old pigs infected with a human isolate of Asian strain influenza virus and on the successful passage of this virus into a second group of pigs. These findings are discussed in relationship to negative results obtained by others when swine sera, collected from apparently healthy animals before and after the recent Asian influenza epidemic, were tested for the presence of complement-fixing and haemagglutination-inhibiting antibodies.

Six "normal" pigs were inoculated intranasally with 1.0 ml of fluid containing approximately 3 200 000 EID_{50} of Asian influenza virus. Clinical evidence of disease was not apparent, but Asian strain virus was isolated from four of the pigs and the development of haemagglutination-inhibiting antibody to A/Asia/Japan/305/57 antigen was detected in all of them. Virus isolated from the first group was inoculated intranasally into another group of six "normal" pigs. Clinical evidence of illness was also absent in this group, but Asian strain virus was isolated from five pigs. Haemagglutination-inhibiting antibody developed in all six pigs but complement-fixing antibody in none.

The authors conclude that the available evidence indicates that swine did not play a significant role in the epidemiology of the Asian influenza epidemic in the USA, and that the Asian strain appears not to have established itself in swine.

Laidlaw (1935) and Shope (1931, 1936, 1938) have presented evidence that the influenza virus probably responsible for the 1918 human pandemic became adapted to swine and caused an illness in these animals clinically and pathologically similar to human influenza. Francis & Shope (1936) and Shope (1937) were able to infect swine experimentally with the human PR8 strain. Shope (1938) also presented serological evidence of two separate occurrences of natural infection with human influenza virus in swine.

Recently influenza again displayed the geographic spread and other epidemiological characteristics of a pandemic (Dunn, 1958; Shope, 1958). Current information on the incidence and distribution of this disease has

* Present address: Department of Health, Division of Preventive Medicine, Honolulu, Hawaii

been made available by the US Public Health Service in the *C.D.C. Influenza Surveillance Reports*, Nos. 1-41. Meyer and his associates (1957) and Jensen (1957) have presented data showing that the pandemic was caused by type A influenza having quite different antigenic composition from other strains isolated in recent years. Jensen (1957) describes the Asian strain as belonging to a new and fourth set of influenza A strains.

Unconfirmed and unpublished information indicated that the Asian strain of influenza virus was isolated from the lungs of naturally infected swine in China shortly after the occurrence of the first human cases.

Limited studies were undertaken, therefore, to determine whether swine were susceptible to experimental infection with Asian strain influenza virus.

Materials and Methods

Two groups of 6- to 8-week-old pigs were selected for the experiment. The first group consisted of six litter mates chosen from a local herd of mixed Duroc and Poland China breeds. The herd was considered to have been raised under standard normal conditions found in the Alabama area, and there was no history of recent infectious disease. The experimental animals were housed in a room with a concrete floor which had not contained any other mammals for several months. They were observed to be in good health for three weeks before experiments were begun. Three complete blood counts were performed and four nasal washings were collected from each pig before virus was given. These pigs were gradually changed from a commercial starter diet which contained 0.038 g of chlortetracycline per pound to a maintenance diet which contained no antibiotics and was utilized throughout the course of the experiments.

The second group of six pigs was selected from the same herd as group I, but they were not litter mates. They were handled in essentially the same manner as the first group. The same quarters were used for both experiments, but there was a three-week interval after removal of the first animals before the second group was purchased. The quarters were thoroughly disinfected between experiments.

The influenza strain A/Asia/AFP/2/57, given to the first group of pigs, was obtained from the Communicable Disease Center Respiratory Unit, which serves as the World Health Organization Influenza Centre, Montgomery, Ala. It had been isolated from a human patient suffering from influenza and was identified as an Asian strain. Each pig was inoculated intranasally with 1.0 ml of allantoic fluid containing approximately 3 200 000 EID_{50}. The virus had had two egg-passages before it was given to the pigs.

Each pig in the second group was inoculated intranasally with 1.0 ml of 1 : 4 dilution of amniotic fluid collected from eggs infected with nasal washings obtained from the first group of pigs. Each dose contained approximately 400 000 EID_{50}.

Blood for serological study was taken from each pig in both groups before virus was administered and at 11, 18 and 25 days after virus was given. An additional blood specimen was collected at 35 days from the second group. All serum samples were stored at −20° C until tested for the presence of haemagglutination-inhibiting antibodies. The pre-inoculation, 25-day and 35-day specimens were also tested for complement-fixing antibodies.

Nasal washings were obtained by injecting 5.0 ml of sterile buffered water (pH 7.6) into each nasal passage while the animal's head was held erect. The water was then allowed to drain into sterile Petri dishes and stored in test-tubes at −70° C until inoculated into fertile hen's eggs. Washings were collected daily from each pig for the first 11 days after virus inoculation.

Blood cell counts were made by the methods described by Coffin (1945) and were performed daily for each pig during the first 11 days after virus was administered.

Standard influenza diagnostic procedures, as outlined by Jensen (1956), were followed with a few minor variations. Chick-embryos 11-12 days old were inoculated by the amniotic route with a 2-inch, 22-gauge (51 mm, 0.70 mm) needle through a small puncture in the shell. A candler was utilized to detect the embryo. Both amniotic and allantoic fluids collected from the first egg-passage were tested for the presence of haemagglutinins and, if found negative, the amniotic fluid was passed a second time by amniotic inoculation of chick-embryos. Third amniotic passages were not attempted.

The non-specific inhibitor encountered in pig and ferret sera in preliminary haemagglutination-inhibition tests was removed by the following method: 1 volume of serum was mixed with ½ volume of trypsin (8 mg/ml in M/10 phosphate buffer) and heated for 30 minutes at 56° C. The serum and trypsin were then mixed with two volumes of a freshly prepared M/90 aqueous solution of potassium periodate and incubated overnight at 4°C. Periodate was neutralized by adding two volumes of 1% glycerol-saline. Chicken serum was treated with periodate only.

Pig sera were absorbed with 2% chicken cells for the removal of haemagglutinins. Prototype viruses and most of the antisera used were obtained from the CDC Respiratory Unit, Virus and Rickettsia Section, Montgomery, Ala.

Results

Neither group of pigs manifested clinical evidence of respiratory illness throughout the course of the experiments. Body temperatures were recorded twice daily for 6-7 days after virus was administered, and once daily for the remainder of the experiment. There was considerable temperature

fluctuation in the first group before virus was given, but this was attributed to handling. Post-inoculation temperatures remained within the normal range (Dukes, 1947) in both groups of pigs.

There was no clear-cut evidence of a general leucopenia. Considerable fluctuation within the normal range (Coffin, 1945) was observed in the daily leucocyte and differential counts of several of the pigs. A reduction of approximately 1000 neutrophiles per cubic millimetre with a corresponding drop in the total leucocyte count was observed in three pigs in group I during the 7th day after virus was given. Influenza virus was isolated from two of these three pigs. An increase in haemagglutination-inhibiting antibody was observed in all three.

Two pigs in the second group displayed a marked reduction of neutrophiles, approximately 3000 per cubic millimetre, with an increase of juvenile forms, 2000–3000 per cubic millimetre, the fourth day after virus was given. This was not accompanied by a general leucopenia. Influenza virus was isolated from both of these pigs and an increase in haemagglutination-inhibiting antibody was observed. Two other pigs in this group had a slight reduction of neutrophiles on the 5th and 8th days after inoculation. Virus was isolated from one of these animals and both responded with a rise in antibody titre. A slight anaemia was observed in group I, but this was corrected by adding iron supplement to the ration.

Virus was isolated six times from group I and 16 times from group II (Table 1). Four individuals of group I and five individuals of group II

TABLE 1. FREQUENCY OF ASIAN STRAIN INFLUENZA VIRUS ISOLATIONS FROM EXPERIMENTALLY INFECTED SWINE

Pig No.		Days after inoculation										
		1	2	3	4	5	6	7	8	9	10	11
Group I	1	—	—	—	—	—	—	—	—	—	—	—
	2	—	—	—	+	+	—	—	—	—	—	—
	3	—	—	—	+	+	—	—	—	—	—	—
	4	—	+	—	—	—	—	—	—	—	—	—
	5	—	+	—	—	—	—	—	—	—	—	—
	6	—	—	—	—	—	—	—	—	—	—	—
Group II	1	+	+	—	—	+	+	—	—	—	—	—
	2	+	+	—	—	+	—	—	—	—	—	—
	3	+	+	—	—	+	—	—	—	—	—	—
	4	—	+	—	+	—	—	—	—	—	—	—
	5	+	—	—	+	+	—	—	+	—	—	—
	6	—	—	—	—	—	—	—	—	—	—	—

TABLE 2. HAEMAGGLUTINATION-INHIBITION TITRES * OF CHICKEN ANTISERA WHEN TESTED WITH VIRUS ISOLATES FROM PIGS

Antisera	Antigens [a]												
	p2	p3	p4	p5	sp1	sp2	sp3	sp4	sp5	A/Asia/Japan/305/57	A/Denver/1/57	B/Great Lakes/1739/54	A/Swine/1976/31
A/Asia/Japan/305/57	320	320	160	160	80	160	160	320	160	320	<10	<10	<10
A/Denver/1/57	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	160	<10	<10
B/Great Lakes/1739/54	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	160	<10
A/Swine/1976/31	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	640
A/Asia/AFP/2/57 p2 [b]	160	160	160	80	80	160	80	160	160	160	<10	<10	<10
A/Asia/AFP/2/57 sp2 [c]	160	320	160	160	160	160	160	160	160	320	<10	<10	<10

* Serum titres are expressed as reciprocal of initial serum dilution.

[a] The designations p2, p3, etc. and sp1, sp2, etc. indicate isolates from pigs of the corresponding number in groups I and II respectively.

[b] Serum from chicken immunized with virus obtained from group I, pig 2

[c] Serum from chicken immunized with virus obtained from group II, pig 2

accounted for these isolations. The isolates were subsequently passed 2–4 times by the allantoic route and identified as belonging to the Asian set of strains by the haemagglutination-inhibition test. The isolates shown in Table 2 were selected as representatives from each pig. There was only a twofold difference in all but one serum titre when the isolates were tested with appropriate immune chicken sera by the haemagglutination-inhibition test; the titres of both groups of experimental pigs are shown in Table 3. No haemagglutination-inhibiting antibody against A/Asia/Japan/305/57 was present in a 1:10 dilution of the pre-inoculation serum from any of the pigs, but such antibody to that strain developed in all pigs after inoculation with virus and in all but three pigs it developed to titres above 1:10. Specific inhibition of A/Denver/1/57, B/Great Lakes/1739/54, and A/Swine/1976/31 antigens was not evident in the sera of any of the pigs. All serum samples were included in the same haemagglutination-inhibition test with the exception of the pre-inoculation sera from pigs 1, 2, 3, 4, 5, and the 25-day serum from pig 4 in group I. Because of earlier difficulty with non-specific inhibition and serum haemagglutinins, there were insufficient quantities available for inclusion in the final test. However, all the pre-infection sera had previously been shown to be free of antibody. Complement-fixing antibody could not be detected in any of the pig sera tested, utilizing both soluble and viral antigens.

TABLE 3. HAEMAGGLUTINATION-INHIBITION TITRES * OF SERA FROM PIGS INFECTED WITH A/ASIA/AFP/2/57 STRAIN OF INFLUENZA VIRUS TESTED WITH A/ASIA/JAPAN/305/57 ANTIGEN

Pig No.		Days after inoculation				
		0	11	18	25	35 [a]
Group I	1	<10 [b]	<10	80	20	
	2	<10 [b]	20	80	40	
	3	<10 [b]	40	40	20	
	4	<10 [b]	not taken	80	40 [b]	
	5	<10 [b]	<20	10	10	
	6	<10	<10	10	10	
Group II	1	<10	20	40	40	20
	2	<10	10	10	10	10
	3	<10	40	20	40	20
	4	<10	40	40	40	40
	5	<10	40	40	40	80
	6	<10	80	80	20	10

* Serum titres are expressed as reciprocal of initial serum dilution. Haemagglutination-inhibiting antibody could not be detected in serum dilutions of 1 : 10 or greater using A/Denver/1/57, B/Great Lakes/1739/54, or A/Swine/1976/31 antigens. These antigens were inhibited by homologous immune chicken serum, but not by heterologous immune chicken serum.

[a] 35-day samples were taken from group II only.

[b] Serum tested at different time.

Discussion

A number of factors influencing clinically observable influenza in swine have been thoroughly discussed by Shope (1931b, 1932, 1938, 1951, 1958; also Lewis & Shope, 1931; Orcutt & Shope, 1935; Jensen, 1957). He found a bacterium, *Haemophilus influenzae suis*, to be associated with the majority of naturally occurring cases of swine influenza. This led to the discovery of a filterable agent which would not produce typical swine influenza unless accompanied by the *Haemophilus* bacillus. He further demonstrated that when swine were given only swine influenza virus, isolated from naturally occurring cases, very mild symptoms, sometimes escaping recognition, occurred with subsequent development of neutralizing antibody and immunity. This syndrome was termed " filtrate disease " and could be transmitted among swine by direct contact. " Filtrate disease " was also produced by inoculating swine intranasally with the PR8 strain of human influenza virus (Shope, 1937). When the inoculum was accompanied by *H. influenzae suis* organisms, the animals developed more obvious clinical symptoms.

In later reports Shope (1943, 1955) discusses the role of swine lungworms harbouring " masked " virus. Typical influenza was produced in swine parasitized with influenza-infected lungworms by provocation with multiple intramuscular injections of the *Haemophilus* bacillus. Intranasal inoculation of human influenza virus or exposure to adverse weather conditions, as well as other stimuli, also occasionally precipitated clinical disease. Provocation was not successful during the summer months.

It seems likely that " filtrate disease " was produced in the twelve pigs used in the experiments being reported. No attempts were made to culture *H. influenzae suis* bacillus from the respiratory tracts of the pigs at any time during the experiments. Lewis & Shope (1931) regularly cultured a haemophilic bacillus from the respiratory tracts of experimentally and naturally infected swine but not from swine free of influenza.

In a later experiment, Shope (1955) was able to produce clinically severe disease in 6 out of 25 " prepared " swine in the absence of concomitant infection with *H. influenzae suis*. These swine had previously been fed earthworms containing lungworm larvae obtained from swine with influenza. He explained this phenomenon by stating that " swine in which virus has been provoked exhibit a more extensive pneumonia and one with a considerably different distribution than do those infected with virus by the nasal route ".

It should be pointed out that the swine in our experiments received no intentional provocative stimulus, but the experiments were conducted during winter months which were described by some as being the worst weather experienced in the region for 40 years. The experimental animals were housed in an enclosure containing no heat and its construction allowed a free flow of outside air. The minimum temperature during the first experiment was 17°F (−8.3°C) and the maximum recorded was 72°F (22.2°C). The average temperature for the 11 post-inoculation days during the second average temperature for the 11 post-inoculation days during the second experiment was 7°F (3.8°C) below normal with minima varying from 19°F (−7.2°C) to 38°F (3.3°C) and maxima from 45°F (7.2°C) to 70°F (21.1°C).

The significance of artificial infection of swine with Asian strain human influenza virus can only be speculative. These animals received a relatively heavy dose of virus which was contained in chick-embryo allantoic and amniotic fluids. No antigenic change was noted in the virus after two porcine passages. There was highly suggestive evidence that some adaptation of the virus to swine occurred after one pig passage, as shown by a 62% increase in virus isolations in the second group of pigs.

Swine sera obtained from various areas of the USA during and after the influenza epidemic have been tested recently for the presence of haemagglutination-inhibiting antibody. To date no serum specimens have contained antibody against A/Asia/Japan/305/57 antigen, while significant titres were

obtained in many instances against the swine influenza virus (R. Q. Robinson —personal communication). It is possible that swine which are immune to swine virus may also be refractory to infection with Asian strain virus (Shope, 1937). With the exception of the possible occurrence in China referred to earlier, there have been no reports of naturally occurring Asian strain infection in swine.

The available evidence indicates that swine did not play a significant role in the epidemiology of the recent influenza epidemic occurring in the United States of America, and it appears that the Asian strain virus has not established itself in swine.

RÉSUMÉ

Les recherches de divers auteurs avaient laissé supposer que le virus grippal responsable de la pandémie de 1918 s'était adapté au porc et avait causé chez cet animal une infection comparable à la grippe. Par ailleurs, dans deux cas, séparés géographiquement, l'analyse sérologique avait révélé chez le porc une infection à virus PR8. Des études ont été entreprises en vue de déterminer si le porc est sensible à l'infection expérimentale par le virus de la grippe asiatique.

La souche de virus grippal A/Asia/AFP/2/57, isolée d'un cas de grippe humaine, et ayant subi 2 passages sur œuf, a été injectée par voie intranasale à un premier groupe de 6 porcs de 6-8 semaines, maintenus en parfaite condition de santé, et sur lesquels on a procédé à des examens sérologiques et hématologiques avant l'infection. Chaque porc a reçu environ 3 200 000 DIE_{50} (dose infectante pour l'embryon) de virus. Chaque animal du second groupe — composé comme le premier — a reçu environ 400 000 DIE_{50} du virus prélevé sur les animaux du premier groupe. Aucun des animaux ne présenta les symptômes d'une infection respiratoire au cours des essais. La leucopénie n'était pas évidente, bien que le nombre des neutrophiles ait diminué chez quelques animaux.

Le virus a été isolé 6 fois du groupe 1, le 2e, le 4e et le 5e jour après inoculation, et 16 isolements ont été effectués sur le groupe 2, le 1er, 2e, 4e, 5e, 6e et 8e jour. Quatre animaux du groupe 1 et cinq du groupe 2 étaient porteurs de virus. Tous les virus isolés appartenaient à la série des virus asiatiques.

Les anticorps inhibiteurs de l'hémagglutination (IHA) ont été décelés au titre de 1:10 ou plus, chez 8 des 11 porcs examinés 11 jours après l'infection. Des titres de 1:10 1:80 ont été notés chez les 12 porcs, le 18e et le 25e jour. Trente-cinq jours après l'injection du virus, des anticorps IHA étaient démontrables chez tous les porcs, mais aucun anticorps fixateur du complément n'a pu être mis en évidence.

Les animaux soumis à ces expériences étaient maintenus dans un local non chauffé, à la fin de l'automne et au début de l'hiver. La température était inférieure de 3,8°-8°C à la température normale, et l'écart entre les températures minimum et maximum atteignait 34°C.

La portée de ces expériences reste conjecturale. Les animaux ont reçu une dose de virus relativement élevée, véhiculée par les liquides embryonnaires du poulet. Aucune modification antigénique n'a été observée après deux passages chez le porc. Il semble pourtant qu'une certaine adaptation se soit produite après le premier passage, car la proportion d'isolements a augmenté de 62% au 2e passage. Des sérums prélevés sur les porcs dans diverses régions des Etats-Unis pendant et après l'épidémie de grippe, ont été examinés pour leur teneur en anticorps IHA. Jusqu'à maintenant, aucun des sérums éprouvés ne contenait d'anticorps contre le virus A/Asia/Japan/305/57, alors qu'ils présentaient des titres élevés d'anticorps anti-grippe du porc. Il est possible que les porcs résistants au virus de la grippe porcine le soient également au virus asiatique.

Il semble, d'après les renseignements réunis jusqu'à maintenant, que les porcs n'aient pas joué un rôle important dans la récente épidémie de grippe aux Etats-Unis, et le virus asiatique ne paraît pas s'être fixé chez cet animal.

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